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Choice and Specificity of Complement in Complement Fixation Assay

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This study examines the relative activity of various complement sources in a variety of antigen-antibody (Ag-Ab) complement fixation assay systems. Studied were Ag-Ab systems of cytomegalovirus, adenovirus, *Pneumococcus*, *Coccidiodes*, and guinea pig kidney. Ab titers in each system were determined by micro-complement fixation assay using two batches of titrated rabbit, monkey, cat, dog, human cord, human adult, and guinea pig sera as complement sources. Assay sensitivity and Ab titers proved to be strongly related to the complement source. There was considerable variation in the ability of Ag-Ab complexes to bind the complement of each of the species tested. Guinea pig, rabbit, and monkey sera gave comparable titers in four of the five Ag-Ab systems tested. Cat serum complement was able to detect anti-adenovirus and anti-*Pneumococcus* reactivity only. Human adult, human cord, and dog sera exhibited anti-complementary activity. The study emphasizes the importance of complement source in determining both the specificity and sensitivity of complement fixation assays in a given Ag-Ab system.

Among the serological assays routinely used to detect bacterial, viral, and tumor antigens (Ags) and their related antibodies (Abs) are complement fixation (CF), cell-dependent microcytotoxicity, and immunofluorescence assays. The first two are always complement-mediated reactions, and the immunofluorescence assay may be made more sensitive by using a complement-dependent technique.

Our laboratory has been focusing its efforts on the detection of human sarcoma-associated Ags by serological means. To date this work has resulted in the discovery of three Ags which are associated with human sarcomas S_1, S_2, and S_3 (4, 6, 9). S_1 and S_2 were detected by immunofluorescence, whereas S_3 is a complement-fixing Ag. While attempting to standardize assay methods for S_3 Abs, we observed that S_3 Abs in human serum samples could best be demonstrated if fresh human cord serum served as the source of complement. High titers were consistently detected with this serum. The same serum was unreactive or gave low titers with guinea pig complement. Rabbit complement batches were found usually to possess anti-complementary activity (10).

To learn more about the influence of the complement source on CF tests, a study was undertaken to examine changes in the specificity and sensitivity of various CF assays for known Ags and their respective Abs when complement from different species is employed.

MATERIALS AND METHODS

Ag-Ab. Cytomegalovirus, adenovirus, and *Coccidioides* Ags and their respective Abs were obtained from Microbiological Associates, Bethesda, Md. *Pneumococcus* strain 8 Ag and the related Ab were obtained through the courtesy of Gerald Schiffman of Downstate Medical Center, Brookyn, N.Y. The guinea pig kidney Ag used was a 20% (by volume) sonicated preparation of fresh guinea pig kidney tissue in phosphate-buffered saline. Ab to this Ag came from a patient with osteosarcoma.

Complement. Except for guinea pig serum (GPS) complement, which was obtained from commercial sources (Microbiological Associates), all other complement types were derived from fresh serum samples prepared from clotted blood specimens centrifuged at 2,000 × g for 30 min at 4°C within 1 h after the blood was collected. The samples were divided into 0.5-ml portions and stored at −70°C in a Revco Freezer until used. Fresh human adult blood samples were supplied from blood donors through the courtesy of the New York Blood Center. Human cord blood was carefully drawn from placentas at the Lying-In Hospital, New York, N.Y. Human adult sera and human cord sera were prepared by centrifugation as mentioned above.

All complements were titrated for complement activity on the same day the CF test was run. For test purposes, each complement sample was diluted with Veronal-buffered saline to provide 2 units of activity in a volume of 0.025 ml (Table 1).

CF assay. A standard micro-CF method was used

TABLE 1. *Serum samples used as sources of complement*

Complement source	Batch no.	Dilution giving 2 units of complement per 0.025 ml
Guinea pig	1	1:40
Guinea pig	2	1:40
Cat	1	1:4
Cat	2	1:2
Rabbit	1	1:2
Rabbit	2	1:2
Dog	1	1:6
Dog	2	1:8
Monkey	1	1:8
Monkey	2	1:8
Human adult	1	1:8
Human adult	2	1:12
Human cord	1	1:6
Human cord	2	1:12

(11). All dilutions were made with Veronal-buffered saline. Ab-containing sera were diluted and then inactivated at 56°C for 30 min. Each experiment included controls for serum, Ag, complement, and sheep erythrocytes (SRBC). The results were recorded on a 0 to 4+ scale corresponding to the size of the erythrocyte button and the amount of complement fixed. A button size of 2 and serum free of any anti-complementary activity were considered as indicating a positive serum. Anti-SRBC serum (Difco Laboratories, Inc., Detroit, Mich.) was pretitrated and freshly diluted for incubation with SRBC. Tests were performed in duplicate the same day.

The CF test was conducted by delivering 0.025 ml of Veronal-buffered saline to each well in the plate. Serum was added to the first horizontal row of wells, and twofold dilutions were made in a vertical direction with 0.025-ml diluting loops. Every serum sample was diluted through two rows of wells, and the remaining wells received a similar volume of Veronal-buffered saline. Finally, 2 100% hemolytic units of complement was added to all wells, and the plates were incubated overnight in a plastic bag at 4°C. The next day, 0.050 ml of sensitized SRBC was added to each well. SRBC were sensitized by incubation with an equal volume of rabbit antiserum against SRBC at 1:800 at 37°C for 30 min. The whole plate was again incubated at 37°C for 1 h with gentle intermittent shaking, and the plate was read after 1 h.

RESULTS

Titers obtained for the five Ag-Ab test systems using 14 complement sources from six species are shown in Table 2. Ab to adenovirus and pneumococcal Ags could be detected by GPS, rabbit, monkey, and cat complement. Anti-cytomegalovirus and anti-coccidioides Abs were detected by GPS, rabbit, and monkey complement. However, anti-guinea pig kidney activity could be determined only with GPS. In all five Ag-Ab systems studied, guinea pig complement gave positive titers. In four of the five systems, rabbit and monkey sera could also be used as complement. Cat serum complement permitted detection of anti-*Pneumococcus* and anti-adenovirus AB activity but gave negative results with GPK, *Coccidioides*, and cytomegalovirus Ag-Ab systems. Human adult, human cord, and dog serum samples generated strong anti-complementary reactivity in many of the Ag control wells.

Although 2 units of complement was used in all reactions, considerable variation in titer occurred even when different batches of complement from the same species were used in the detection of a single Ag-Ab system. As seen in Table 2, in the cytomegalovirus system serum complement from rabbit 1 gave the highest titer, but rabbit 2 serum complement produced a negative titer. Variations in anti-adenovirus titers were also observed with the two batches of rabbit serum complement. Two different batches of guinea pig complement also detected variable Ab titers in the *Coccidioides*, adenovirus, and *Pneumococcus* Ag-Ab systems.

DISCUSSION

GPS has long been used as a source of complement in CF assays (8). The CF assay is widely used in viral immunodiagnostic studies; GPS is the complement of choice in most viral Ag-Ab systems (1). Huebner has utilized the CF assay technique extensively in his studies related to viral and tumor antigens (5). The complement source used in his assays was also primarily GPS. There are reports in the literature, however, in which complement from other species in certain Ag-Ab systems has been found to be more effective and appropriate. Transplantation Ag studies by Colombani et al. using the CF method reveal that the use of human adult serum instead of GPS as a complement source gave better results in human histocompatibility antigen serology (2). Eilber and Morton, while working on human sarcoma-specific Abs, demonstrated that certain antitumor Abs only fixed human complement (3). For determinations of anti-S_3 titers, we have discovered that there is a need for human cord or adult serum as complement source (10). In the light of these findings about the influence of complement source, comparing binding of GPS complement to Ag-Ab complexes in a variety of Ag-Ab systems with that of complement from other sources, therefore, is of vital interest to a serologist.

It seems that the nature of the Ag-Ab complex and the proper selection of complement source determine a positive reaction. The standard Ag-Ab complex used to quantitate the level of complement and its components is SRBC and anti-

TABLE 2. *Titers of Ab to various Ag systems using different sources and batches of complement*

Complement source	Ab titer in Ag system				
	Guinea pig kidney	*Coccidioides*	Cytomegalovirus	Adenovirus	*Pneumococcus*
Guinea pig 1	1:8	1:32	1:32	1:128	1:8
Guinea pig 2	1:16	1:8	1:32	1:32	1:64
Rabbit 1	AC[a]	1:64	1:64	1:16	1:16
Rabbit 2	AC	1:64	–[b]	1:128	1:32
Monkey 1	AC	1:64	1:32	1:128	1:16
Monkey 2	AC	AC	1:32	1:128	1:32
Cat 1	–	–	–	1:64	1:64
Cat 2	–	–	–	1:16	1:32
Dog 1	–	–	AC	AC	AC
Dog 2	–	–	AC	AC	AC
Human cord 1	AC	AC	AC	AC	AC
Human cord 2	AC	AC	AC	AC	AC
Human adult 1	AC	AC	AC	AC	AC
Human adult 2	AC	AC	AC	AC	AC

[a] AC, Anti-complementary activity.
[b] –, Negative.

SRBC. Our studies demonstrate that binding of complement to this standard immune complex does not correlate with the binding of complement from different sources in a test Ag-Ab complex. There was a considerable variation in the ability of different Ag-Ab complexes to bind the complement of each of the species tested. Species-specific utilization of complement has been reported by Perryman and co-workers (7). They were unable to detect Ab to porcine gamma globulin and bovine fibrinogen in cats by using GPS as the complement source. The Ab could be detected, however, when feline serum was utilized as the complement source.

In our test systems human cord, human adult, and dog sera, when used as complement sources, were found to induce anti-complementary activity in the Ag control wells. This could be attributed to species incompatibilities, lipid components, or other complement-binding proteins present in the serum. Selection of a proper complement type may eliminate this problem.

Although the number of sera used as complement sources is limited, this study demonstrates the importance of choice of an appropriate source of complement for optimal determinations of Ab titer to a particular Ag-Ab system.

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